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A PHYSIOLOGICAL AND HISTOLOGICAL STUDY OF THE MEDULLOPONTINE PATHWAYS OF VAGAL FIBERS INVOLVED IN CARDIAC REGULATION

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled A PHYSIOLOGICAL AND HISTOLOGICAL STUDY OF THE MEDULLOPONTINE PATHWAYS OF VAGAL FIBERS INVOLVED IN CARDIAC REGULATION submitted by Lois Catherine Ellsworth in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

Twenty cats were subjected to decerebration and spinal section to obtain information about the effects of successive rostrocaudal transections on cardiac regulation of these "isolated medulla" preparations in which most sympathetic influence was removed. The consequent changes in heart rate, in duration and magnitude of the reflex bradycardia produced by phenyldiguanide (PDG) and by nor-epinephrine were studied after each transection.

The cats of this series showed a successive increase in heart rate as the brain stem was transected in a caudal direction. The PDG-induced bradycardia and the secondary bradycardia due to nor-epinephrine disappeared after a transection at the level of the rostral tractus solitarius.

Twenty-seven cats were operated on to produce a lesion in the nucleus solitarius. An attempt was made to trace the resultant degeneration of secondary cardiovascular afferents. Only two cats yielded usable results. Degeneration was found in several loci, but only those degenerated fibers in the nucleus intercalatus could be considered to include secondary cardiovascular afferents.

The fact that degeneration was absent above the rostral one-third of the tractus solitarius, together with the disappearance of the PDG reflex bradycardia at the same level, indicates that some secondary cardiovascular afferents synapse in this area but that the activation of such synapses depend upon more rostral pathways.

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INTRODUCTION

There are areas within the lower brain which influence several aspects of cardiovascular function. These central mechanisms can exert this control through several channels; for example, the autonomic pathways to the heart, the sympathetic vasoconstrictor fibers to the vasculature, the sympathetic cholinergic preganglionic fibers to the adrenal medulla and the sympathetic cholinergic vasodilator pathways to the vessels supplying skeletal muscles. However, uncertainties exist concerning their exact location and functional significance.

Active vasomotor points, both pressor and depressor, have been located precisely using stimulation techniques (Bach, 1952, Lindgren and Uvnäs, 1953, 1954). But little attempt has been made to incorporate them into definite pathways or "centers". The vasotonically active areas, demonstrated by transection experiments (Alexander, 1946, Hoff, Breckenridge and Spencer, 1952) give a broad idea of brain stem cardiovascular organiza-

tion, but their perimeters are ill-defined.

Histological studies provide precise description of cardiac pathways but only a few authors (Gerebetzoff, 1939, Dell and Olson, 1951) have attempted to follow the pathways past the first synapse.

The present research was undertaken in the hope of clarifying some of these uncertainties, particularly in regard to the pathways of vagal secondary afferents and the levels of organization of these pathways as they are involved in cardiac regulation.

HISTORICAL REVIEW

The search for circulatory centers within the brain stem and pons was carried out in 1871 by Owsjannikow. He noted a step-wise fall in arterial pressure as he progressively transected the brain stem. He concluded that he had mapped the extent of the vasotonic center. Today, it is known that these circulatory areas are influenced by higher centers. But the method of Owsjannikow, along with stimulation experiments, has been employed in many efforts to catalogue medullopontine vasotonic areas.

Ranson and Billingsley (1916) used stimulating electrodes to locate cardiovascular points in the brain stem of cats. They found a depressor locus in the area postrema and a pressor point at the apex of the ala cinera. They did not come to a conclusion about the nature of these points, but stated two possibilities -- that they were stimulating the afferent limb of a cardiovas-

cular reflex arc or that the points represented true vasotonic centers. The same reaction was obtained by Scott (1925) and Wang and Ranson (1939). The latter workers found vasomotor areas in cats, in the lateral reticular formation, adjacent to the floor of the fourth ventricle. Stimulation here produced either a rise or fall in blood pressure, although depressor responses were more numerous in the region of the vagal nuclei.

Alexander (1946) presents a slightly different picture of vasotonic regions. He located a pressor region rostral to the auditory tubercles in cats. Transection through these structures led to a considerable fall in blood pressure together with a reduction of activity from the inferior cardiac nerve. These results received further support from the work of Bach (1952). He stimulated the floor of the fourth ventricle in cats and found a large concentration of pressor points in an area which corresponds to Alexander's pressor region.

Unlike Alexander, Lindgren and Uvnäs (1953, 1954) were concerned primarily with the effect of brain stem stimulation on peripheral vascular tone. Their depressor points (in cats and dogs) are described as being in the same area as those of Wang and Ranson (1939). Stimulation

of this depressor area was accompanied by vasodilation. Since no vasodilator nerves were involved, the dilatation of the vessels was due to inhibition of vasoconstrictor tone.

Rosen (1961) confirmed the findings of Lindgren and Uvnäs (1953). He also confirmed the results of Lindgren et al. (1956) by showing that stimulation of the ventrolateral medulla in cats produced pressor responses (tachycardia and an increase in cardiac contractile force) along with vasodilation. The vasodilation was abolished by atropinization and thus he concluded that he was stimulating sympathetic vasodilator nerves.

Active vasomotor participation concurrent with pressor responses to brain stem stimulation was also demonstrated by Manning (1965). He evoked pressor responses throughout the diencephalon and brain stem of cats that were adrenalectomized, vagotomized and artifically ventilated. These pressor responses were always accompanied by vasomotor reactions.

Thus, pressor and depressor areas have been found in the area postrema, ala cinera, the area rostral to the auditory tubercles, the medial part of the medulla, the ventrolateral portion of the medulla and the region of

the vagal nuclei. Stimulation of some depressor points (for example, those in the area postrema) results in vasodilation (caused by inhibition of vasoconstrictor tone) while stimulation of the ventrolateral medulla activates sympathetic cholinergic vasodilator nerves.

There has been a concern with the effects of pressor and depressor centers on heart rate alone. Hoff, Breckenridge and Spencer (1952) performed transections on decerebrate dogs and found that a mid-pontine transection resulted in marked bradycardia. The fall in heart rate was not relieved by a section through the lower-most third of the pons but was abolished after a transection through the auditory tubercles or by vagotomy. These results were repeated by Glasser (1962) in cats. Because a lower pontine section did not abolish the bradycardia, the mid-pons could not be a primary reflex center. In the words of the authors:

> ... the vagal preponderance of the mid-pontile animal must result from impulses descending from the pons to the primary reflex center and does not represent the unchecked tendencies of that center itself.

The influence exerted upon medullopontine cardiovascular areas by higher centers is more difficult to define than is the influence of these centers on areas

within the lower brain. Thompson and Bach (1950) found that stimulation of the medial reticular formation in cats (a depressor region) reduced hypertension induced by hypothalamic stimulation. A lesion in the former area enhanced the rise in blood pressure produced by stimulation of the lateral portion of the posterior hypothalamus. Thus impulses from medullary depressor fibers were converging upon multiple sympathetic pathways descending from the hypothalamus.

Some of these efferent pathways may be located in the ventrolateral medulla. Peiss (1960) found that stimulation of this area produced an 80% rise in blood pressure in chloralosed cats but only a 15% rise in cats under pentobarbitol anaesthesia. The two anaesthetics affect the hypothalamus in different ways. Peiss felt that this was evidence for efferent hypothalamic pathways in this medullary region.

Activation of depressor tracts or nuclei included in hypothalamic pathways was thought to be the reason for the intense (potentially lethal) hypotensive response found by stimulation of the ventromedial medulla (Tuttle and Conner, 1965), because Folkow et al. (1959) showed that this same type of response could be produced upon

selective stimulation of the hypothalamus.

That autonomic pathways in the lower brain are integrative in function was postulated by Salmoirhagi (1962). He found only one type of "cardiovascular neurone" in the pontine area and its activity was not rhythmic. Because of this, the author feels that such neurones are receiving and integrating impulses from several sources. Supramedullary tonic influence has also been demonstrated by Reis and Cuenod (1965) who found a drop in basal heart rate and an augmentation of the reflex drop in blood pressure (due to stretching the carotid sinus) after midcollicular decerebration.

Structural Organization Of Medullary Centers Probably Involved in Cardiovascular Regulation

It is evident that cardiovascular "centers" within the brain stem and pons are influenced by and integrated with those of higher centres. But quantitative measurement of this higher influence is not available nor are the pathways adequately described.

Taber (1961) describes the extent of the nucleus solitarius as follows:

> ... from the level of pyramidal decussation to the level of the caudal pole of the nucleus vestibularis lateralis. At the level of the obex, the nucleus has a

small medial extension and the large mid-portion of the nucleus is located ventral to the enlarged area postrema, directly ventral to the floor of the fourth ventricle.

Degeneration within the solitary tract and its nucleus has been observed after section of the Vth and VIIth cranial nerves (Schwartz et al., 1951, Torvik, 1956) and after section of the IXth and Xth cranial nerves (Foley and Dubois, 1934, Ingram and Dawkins, 1945, Schwartz et al., 1951, Torvik, 1956 and Cottle, 1964). The efforts of these workers have produced the following results:

a. Some afferent facial, glossopharyngeal and vagus nerves terminate (in the nucleus solitarius) in a successive rostrocaudal manner. But the boundaries are not clear. For example, Cottle (1964) found that the primary IXth afferents end in the rostral and intermediate regions of the nucleus solitarius while the Xth afferents end mostly in the intermediate portion.

b. The nucleus is most often divided into:

1. A ventral region which is ill-defined and is composed of large and medium sized cells,

2. a medial region, consisting of a dense population of small cells,

3. a lateral portion which is ill-defined and is composed of large cells,

4. the commissural nucleus of Cajal.

c. Degenerated fibers from the IXth and Xth nerves enter the solitary tract both laterally and ventrally to pass caudally. However, the termination of these fibers in the nucleus solitarius is at approximately the same level as that at which they leave the tractus.

d. Degeneration within the nucleus usually is associated with degeneration in the commissural nucleus of Cajal. The one exception to this is found in the results of Schwartz et al. (1951).

According to Dell and Olsen (1951) the secondary vagal afferents may ascend to the thalamus where there are synaptic connections in the ventro-medial thalamic nuclei. The morphological study of Gerebetzoff (1939) has also implicated the thalamic nuclei as receiving secondary vagal afferents.

Thus, it is likely that the primary cardiovascular afferents are contained within the tractus solitarius and its nucleus. Stimulating techniques have lent support to this statement. Stimulation of the vagus has resulted in the recording of evoked potentials in and around the

solitary tract (Urabe, Takashi, and Tsubokawa, 1960 and Porter, 1963). Calaresu and Pearce (1964) produced bradycardia and hypotension upon stimulation of the tractus solitarius in cats. Compound action potentials, which followed the stimulus frequency exactly were recorded upon stimulation and therefore, the authors felt that the vagal activity was due to "antidromic discharges in afferent fibers".

In summary, although the path of the primary cardiovascular afferents is precisely known, the route of the secondary afferents is largely unknown and this justified our attempt to study these pathways histologically. The earlier part of the literature review indicates the uncertain state of knowledge of the functional integration of brain stem regulation of cardiovascular activity, and the contribution of the parasympathetic system to cardiac control was selected for further physiological study.

METHODS

Isolated Medulla Series

Twenty cats ranging in weight from 1.4 to 3.8 kg. were anaesthetized by open ether inhalation following induction with ethyl chloride. A tracheal cannula was inserted and the carotid arteries exposed, isolated and tied loosely with loops of thread. Either the right or left femoral artery and vein was catheterized. The rectal temperature was kept between 37°C and 39°C by using a heating pad with three controls which was placed under the cat. The animal was then fixed in a stereotaxic apparatus fitted with a micrometer. The occipital bone was exposed and partially removed and any sources of bleeding from the bone were plugged with plasticine. The cerebellum was removed by suction after which post-tentorial mid-collicular decerebration was carried out with the aid of a small, blunt spatula. During this procedure, the carotid arteries as well as the vertebrals were occluded. The anaesthetic was discontinued following

decerebration.

After allowing the animal to recover from the above procedures, a spinal section was performed. The arch of the atlas and the meninges were removed at the level of C_1 - C_a at the same time as the above procedures. The cord was sectioned with the use of a spatula. Immediately after spinal section, artificial respiration was initiated with a Palmer pump. The exposed brain stem was then covered with mineral oil* to prevent drying.

Arterial pressure in the femoral artery was recorded with a Statham P23-A strain gauge, the input to which was a heparinized, saline-filled catheter (placed in the femoral artery). The output was connected to either (a) one of the beams of a Tetronix 502 dual beam oscilloscope via a channel of an Electronics for Medicine polygraph or (b) one of the channels of a conventional pen recorder (Offner Beckman type RB Dynograph). If the oscilloscope was used then the arterial pressure was recorded on either Kodak type or Ilford NS6 recording paper using a Dumont 321-A camera.

Drugs were injected intravenously via the femoral

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* a liquid paraffin

vein catheter. PDG* (an amine derivative which elicits reflex bradycardia and hypotension), epinephrine and occlusion of either or both the common carotid arteries were the drugs and physiological procedures respectively used to evoke cardiovascular responses.

Transections of the brain stem were done at approximately 8mm, 6mm, 4mm and 2mm above the obex using a sectioning device made out of a small piece of razor blade in a holder. The holder was attached to the micrometer which could be moved laterally, crainiocaudally and vertically in steps of 100μ. The more rostral transections (8mm and 6mm) traversed the whole depth of the brain stem while any caudal to these were usually at a depth of from 2 to 4mm below the surface of the fourth ventricle. All the transections extended across the entire width of the brainstem but the intracranial rootlets of the Xth nerve were left intact. After each transection, cardiovascular responses to PDG,** epinephrine and stimulation of the carotid baroreceptors were recorded. At the termination of the experiment, the animals were perfused via the abdominal aorta, initially with a solution of 0.9% NaCl and then with 10% formalin in 0.9% NaCl. The brain stem was removed and stored in

* Phenyldiguanide
** Dosage = 100μg

10% formalin.

The brain stem was sectioned at 50μ intervals with a Leitz freezing microtome and sections rostral and caudal to each transection were studied to determine its exact level. The sections were mounted on glass slides using Mayer's egg albumin (three drops to 20ml of water) and allowed to dry overnight in an oven kept at 36-37^{o}C. Following this, the slides were successively immersed in an ascending series of water and alcohol mixtures (ie., increasing concentration of alcohol), cleared in a 1:1 mixture of ether and chloroform for 2-5 minutes, allowed to dry in the air and then hydrated. They were then stained for three minutes in Kernechtrot (100mg nuclear fast red dissolved in a 5% aluminum sulphate solution), dehydrated and covered. The stained material was examined microscopically to determine the level of each transection, especially its relation to the tractus solitarius and the dorsal motor nucleus of the vagus.

Partial Transection Series

Fourteen cats ranging in weight from 1.8 to 4.5 kg were anaesthetized with chloralose (60-80 mg/kg. injected intravenously) after induction with ethyl chloride and

ether. The skin and muscle overlying the brain stem were retracted and the occipital bone removed. The cerebellum was removed by suction and transections of the brain stem were then carried out using either a small piece of razor blade or a small steel rod filed down to a sharp, thin point. This sectioning device was fixed to a stereotaxic apparatus in the same manner as described in the "Isolated Medulla Series." The ECG was displayed on a Nagard oscilloscope after being fed through a Grass P-5 differential amplifier and recorded by a Dumont 321-A camera on Ilford NS6 recording paper.

Partial transections were made at different levels of the bulbar region in an effort to produce a severe bradycardia (50% or more reduction from the initial heart rate). It was thought that this method would yield more precise results than complete transections and thus small pressor or depressor areas could be located. Any anatomical information gained on reactive cardiovascular areas might then be correlated to the histological findings concerning secondary afferents arising from the nucleus of the tractus solitarius.

Sections were made in the following regions:

1. From 10 to 2mm above the obex on either the

right or left side of the fourth ventricle.

2. From 10 to 2mm above the obex within the fourth ventricle and excluding the lateral areas of the brain stem.

3. Sections running longitudinally in the region of the mid-line of the fourth ventricle.

Each of the above types of transections was done in dorso-ventral stages. Thus heart rate was recorded at 2mm below the surface, 3mm and so on. At the close of the experiment the animals were perfused via the abdominal aorta, first with 0.9% NaCl followed by 10% formalin in 0.9% NaCl. The brain stems were then removed and stored in 10% formalin.

The nervous tissue was examined in two ways:

1. The whole brain stem was examined under a binocular microscope and the level of each section determined. The depth of the sections were also approximately determined by slicing through the section with a razor blade and measuring the depth of blood infiltration.

2. Fiftyμ sections were cut on a Leitz freezing microtome and stained with nuclear fast red (Kernechtrot) and the sections above and below the cuts studied in order to ascertain the exact level of section in relation

to surrounding structures.

Histology and Lesions of the Tractus Solitarius

Initially, the object of this series was to locate in the nucleus of the tractus solitarius the area described by Calaresu and Pearce (1964) which, when stimulated, produced reflex bradycardia. When this area was found, a lesion would then be placed in the same spot.

Twenty-seven cats were used in this series, ranging in weight from 1.5 to 3.1 kg. Nine cats, comprising the first sub-series, were anaesthetized with Chloralose (60-80mg/kg) following ethyl chloride and ether induction. The skin and muscle overlying the lower brain were then retracted, a small opening was made in the occipital bone and the cerebellum was gently lifted up with the aid of a glass spatula. Bi-polar, stainless steel micro-electrodes, made from insect pins according to the method of Green (1958) and insulated to the tips with INSL-X33 were used for stimulation. The tip diameter was between 10-50μ and the electrodes were approximately 300μ apart. The electrodes were connected to a Grass S-IU4 stimulator through a Grass S-IU4 isolation unit and stimulation was carried out for 5-10 seconds, with a square wave (amplitude 10-15V, duration .1msec,

frequency 20/sec.). The ECG was monitored on a Tetronix 502 dual beam oscilloscope. If bradycardia was elicited, then the electrodes (without being moved from their position in the brain stem) were connected to a Grass lesion maker and a lesion produced by using an intensity of .25-.5V for 10-20 sec.

The animals were allowed to recover and later sacrificed for histological study after 7-10 days, at which time the animal was perfused with saline and formalin as described in the foregoing sections. The brain stem was sectioned at 35μ intervals on a Leitz freezing microtome and every sixth section was stained according to the method of Nauta (1957) in order to trace the degeneration resulting from the lesion. Another series of every sixth section was stained with Kernechtrot (according to the method described under "Transection Experiments") in order to localize the lesion with regard to surrounding tracts and nuclei and to get an accurate idea of the extent of the lesion. In these first nine animals, however, it was found that the lesions, although placed within the nucleus of the tractus solitarius, were much too large. Thus, not only was part of the nucleus destroyed, but also surrounding structures were damaged to a great extent. Because of this, accurate

information concerning the pathways of secondary cardiovascular afferents alone could not be obtained.

The next seven cats were subjected to lesions of the tractus solitarius following the preparation of three control cats in which a series of lesions was placed on either side of the fourth ventricle. Different combinations of intensity and time were used in these control experiments in order to obtain the appropriate parameters for producing a small, discrete lesion. These animals were sacrificed immediately, perfused with the method described for the other types of experiments and the brain stems stored in 10% formalin. Fifty μ sections were obtained using the aforementioned method and the tissue stained with Kernechtrot. It was found that an intensity of .05V for three seconds gave the small lesion that was desired. After histological examination of this second sub-series, it was found that although the lesion was small enough there was still a large amount of degeneration which confused any attempt to follow the course of degenerated fibers from the nucleus of the tractus solitarius. It was concluded that exposure of the brain stem and the stabs with the needle electrodes had produced the unwanted degeneration. Therefore, for the

last eight cats the lesion was placed without testing for bradycardia. In order to expose the brain stem as little as possible, the electrodes were manipulated free-hand. This eliminated the time used in placing the electrodes according to stereotaxic coordinates. The histological examination was carried out in the same manner as described for the first subseries, Nauta and Kernechtrot both being used.

RESULTS

Isolated Medulla Series

Of the twenty cats used in this series, only ten yielded results. Trauma, resulting from decerebration together with spinal section led to death in six animals; a further four animals which survived the above procedures failed to exhibit reflex cardiovascular responses.

A. Basal heart rate

Spinal section was followed invariably by a sharp drop in basal heart rate; however, as the brain stem was transected in a rostrocaudal direction there was always a successive increase in heart rate, save in two instances in which it decreased after transection at the next lower level (Table 1).

B. Change in heart rate with PDG

The magnitude of the PDG-induced bradycardia was approximately the same in the decerebrate, and the decerebrate-spinal animals. This reflex decrease in

heart rate was also evident after transections at 8mm and 6mm above the obex; but there was no trend, to either an increaseor decrease in the intensity of the bradycardia (Table 2).

Histological examination of the brain stems showed that in all cases, the transection which abolished the PDG response was at the level of the rostal 1/3 of the tractus solitarius and just rostral to the dorsal motor nucleus of the vagus (fig.1 and fig. 2).

C. Duration of the reflex bradycardia induced by PDG

With one exception, the duration of the bradycardia, induced by PDG progressively decreased from the decerebration level down to a transection through the rostral tractus solitarius (Table 3).

D. Mean Arterial Pressure

Of the ten cats yielding results in this series, arterial pressure was recorded for nine. The mean arterial pressure followed the same trend as the basal heart rate until a transection at 6mm above the obex was made. After the section at this level approximately half the animals showed a decrease in blood pressure while the other half showed an increase. The section which abolished the response to PDG usually caused a sustained fall in

TABLE I - VARIATIONS IN BASAL HEART RATE FOLLOWING BRAIN STEM TRANSECTIONS AT DIFFERENT LEVELS

	BASAL HEART RATE (beats/min.)				
Cat #	Decerebration	Spinal Section	Section at 8mm Above Obex	Section at 6mm Above Obex	Section at about 4-5mm Above Obex
1	244	187	202	202	164
3	248	142	150	**	
5	240	180	210	135	180
7	*	*	202	225	262
9	225	135	187	225	247
13	255	135	150	172	187
15	160	120	120	170	240
16	200	130	130	150	170
17	204	120	127	135	**
19	220	150	180	180	180
Average	222	145	166	177	204

* Not Recorded - Camera Failure
** Cat Died

TABLE II - DROP IN HEART RATE FOLLOWING ADMINISTRATION OF 100γ of PDG

DROP IN HEART RATE FOLLOWING PDG

(Expressed as Percentage of Basal Heart Rate)

Cat #	Decerebration	Spinal Section	Section at 8mm Above Obex	Section at 6 mm Above Obex	Section at about 4-5mm Above Obex
1	72.9%	65.7%	74.2%	74.2%	54.2%
5	59.6	58.5	57.1	39.2	0
7	*	*	48.0	39.5	33.0
9	52.8	54.6	51.9	50.1	40.0
13	41.2	39.2	35.3	38.9	36.8
15	15.6	16.6	27.5	45.3	47.5
16	40.0	69.1	23.1	26.6	0
17	48.0	43.5	23.6	0.0	**
19	*	61.3	57.8	55.5	50 %
Average	47.1%	51.1%	44.3%	46.2%	45.0%

* Not Recorded - Camera Failure
** Cat died

blood pressure. (Table 4).

E. Effects of Norepinephrine and stimulation of the carotid baroreceptors

Stimulation of baroreceptors by the injection of norepinephrine was used in preliminary experiments. But this led to violent changes in arterial pressure which caused abnormal bleeding and furthermore, the secondary bradycardia produced by norepinephrine was abolished when the PDG response disappeared; therefore the use of norepinephrine was discontinued, and only PDG was used for the rest of the experiments. It should be noted that:

1. The initial hypertensive response to norepinephrine was observed after transection at every level. It was also present after the PDG response had been abolished.

2. The secondary bradycardia was present after every transection until the transection which produced the disappearance of the PDG response.

3. The initial rise in arterial pressure increased further as more transections were made in the caudal direction. (Table).

Mechanical stimulation of the carotid barorecept-

TABLE III - VARIATIONS IN DURATION OF PDG-INDUCED BRADYCARDIA FOLLOWING BRAIN STEM TRANSECTIONS AT DIFFERENT LEVELS in sec.

Cat #	Decerebration	Spinal Section	Section at 8mm Above Obex	Section at 6mm Above Obex	Section at about 4-5mm Above Obex
1	21.6		24.3	13.5	8.1
5	14.8		6.7	5.4	0
7	*		10.8	5.4	6.7
9			13.5	9.4	5.4
13	9.5		8.0	6.6	6.6
15	15.0		9.0	8.0	6.0
16	8.0		6.0	4.0	0
17	12.0		4.0	0	**
19	____		7.0	6.0	6.0
Average	13.5		9.9	7.3	6.0

* Not recorded - camera failure
** Cat Died
Blank spaces indicate that the time was not recorded.

TABLE IV - VARIATIONS IN MEAN ARTERIAL PRESSURE FOLLOWING BRAIN STEM TRANSECTIONS AT DIFFERENT LEVELS mm Hg

Cat #	Decerebration	Spinal Section	Section at 8mm Above Obex	Section at 6mm Above Obex	Section at about 4-5mm Above Obex
1	220	190	119	54	137
5	197	73	113	130	77
7	*	*	76	76	57
9	90	53	76	73	60
13	127	73	78	90	73
15	67	40	57	72	72
16	100	73	67	52	45
17	77	30	28	37	**
19	70	40	60	40	37
Average	118	72	74	69	70

* Not Recorded - Camera Failure
** Cat Died

ors was also discontinued in later experiments because, as with norepinephrine, the response disappeared following a transection through the rostral 1/3 of the tractus.

Partial Transection Series

These experiments were designed to reveal discrete, reactive areas of the brain stem using partial transections; they were later replaced by the Isolated Medulla series, since it provided findings which were more positive. Of the fourteen cats in this series, results were obtained from eleven.

A transection at 7mm above the obex, produced severe bradycardia in four cats (a decrease of 50% or more from the basal heart rate). The slowing lasted for several seconds before returning to a rate which was 80-85% of the rate before transection. These transections extended from 2-5mm lateral to the mid-line and the slowing did not appear till the sectioning device had reached a depth of 4mm. The bradycardia was evident only after a transection on the left hand side of the brain stem; the lack of a response at the same level on the right hand side was probably due to mechanical difficulties in moving the sectioning device laterally.

Partial serial transections progressing caudally from approximately 5mm above the obex, produced a steady increase in heart rate which was persistent. This reaction was evident after transections on either side of the brain stem.

If a mid-line incision was made first, then the bradycardia produced at 7mm above the obex did not appear. Also, the steady rise in heart rate resulting from the more caudal sections was not great and in some cases not evident at all. The mid-line incision was always made in a rostrocaudal direction at a depth of 3-5mm.

Histology

Sub-Series A

Stimulation produced bradycardia in the region of the tractus solitarius in five of the nine cats used. A fall in heart rate was elicited on either the right or left side from 1-2mm rostral to the obex and 1.75-2.5mm lateral to the mid-line at a depth of 1-2mm. In those animals in which bradycardia could not be obtained, a lesion was placed within the area defined by the above parameters. (fig. 3).

In all the animals, degeneration was found in the

nucleus cuneatus, nucleus gracilis, hypoglossal nucleus, dorsal motor nucleus of the vagus and the medial lemniscus. Therefore, although the lesion was placed in the tractus and its nucleus, it destroyed much of the surrounding area in addition. (fig. 4). It was impossible to trace the degeneration resulting from damage only to the tractus or its nucleus.

Control Series

From control experiments, parameters were determined for the lesion-making current which would produce a small, discrete lesion.

It was concluded that .04-.05V for 3-5 seconds would be suitable for producing an appropriate lesion. (fig. 5).

Sub-Series B

Of the next five cats subjected to stimulation, only two exhibited bradycardia; lesions were placed in the areas where bradycardia had been produced in previous experiments. Although the lesion now appeared small enough (fig. 6), degeneration was still profuse in other structures, especially those which lie near the dorsal surface of the brain stem. Degenerated fibers also surrounded the electrode penetrations. In cases where bradycardia was not elicited, degeneration was more

extensive, probably because of the numerous electrode penetrations.

Sub-Series C

In eight cats the lesions were placed free hand in order to expose the brain stem as little as possible. Only two animals in this set showed degeneration in the tractus or its nucleus.

In one of these, BC26, using the method of Nauta (1957) fine fiber degeneration could be traced from the commissural nucleus of Cajal* to the nucleus of the tractus solitarius (figs. 7, 7a and 7b). No degeneration could be traced, on either side, to the dorsal motor nucleus of the vagus, which, at this level, lies just under but slightly medial to the nucleus solitarius. The nucleus intercalatus showed degeneration in this area also, and the degenerate fibers could be traced into the ventral commissure of the cord (figs. 8, 8a and 8b). Dorsolateral to the vagal sensory nucleus and dorsal to the solitary fasciculus, degeneration was observed in the nucleus gracilis. This degeneration followed a dorso-medial direction as the fibers became incorporated into the internal arcuate fibers (figs. 9, 9a and 9b).

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* The mid-line connecting component of the nuclei solitarii.

Just rostral to the level of the obex, at the caudal end of the inferior olivary nucleus, fine, sparse degeneration was seen again in the nucleus solitarius. These fibers were just dorso-medial to the tractus solitarius and curved around its upper extremities. Since so few fibers were involved it was not possible to distinguish their direction outside the area of the tractus. Degeneration was also observed at this level in the nucleus intercalatus and again it seemed to be connected with the ventral commissure of the cord. (figs. 10 and 10a). Fairly dense degeneration was present in the nucleus gracilis and the most dorsal region of the medial lemniscus.

At a level through the upper end of the hypoglossal nucleus, where the dorsal motor nucleus lies medial to the tractus solitarius, almost no degeneration was observed in the nucleus of the tractus. The same situation existed in the nucleus intercalatus and only sparse degeneration could be observed in the medial lemniscus. The rostral portion of the tractus was free of degeneration as was the nucleus intercalatus at the same level. The reticular formation showed no degenerating fibers at any level. However, since degeneration was so sparse,

some small amount could have been overlooked.

Some of these findings were duplicated in BC22, the other animal in which a lesion of the tractus was involved. However, fixation of this brain stem was poor and resulted in poor staining.

In summary, the Isolated Medulla Series yielded the following results:

1. A successive increase in basal heart rate as the level of transection approached the obex.

2. Disappearance of the reflex response to PDG (ie. bradycardia and hypotension) following a section rostral to most or all of the dorsal motor nucleus of the vagus and just caudal to the rostral 1/3 of the tractus solitarius.

3. Disappearance of the reflex bradycardia and hypotension due to stimulation of the carotid baroreceptors and elimination of the secondary bradycardia after administration of norepinephrine after transection at the same level mentioned in (2).

4. In general, a shortening of the duration of the reflex bradycardia induced by PDG as transections were made at progressively lower levels of the brain stem.

Histological results were as follows:

1. only two animals in all the series yielded positive data about degeneration of secondary cardiovascular afferents.

2. At the level of the obex, this degeneration was observed in:

(a) the commissural nucleus of Cajal (to the nucleus solitarius)

(b) the nucleus gracilis

(c) the nucleus intercalatus

(d) the ventral commissure of the cord

(e) the internal arcuate fibers

3. At a level near the caudal end of the inferior olivary nucleus, degeneration was observed in:

(a) the nucleus solitarius

(b) the nucleus intercalatus

(c) the ventral commissure of the cord

(d) the medial lemniscus

4. At a level through the upper end of the hypoglossal nucleus very sparse (almost none) degeneration was observed in:

(a) the nucleus solitarius

(b) the nucleus intercalatus

(c) the medial lemniscus

5. The level through the rostral end of the tractus solitarius did not exhibit any degeneration.

6. No degeneration was seen in the reticular formation or the dorsal motor nucleus of the vagus.

The partial transection series gave little information except for:

1. the severe bradycardia observed after a lateral, partial transection at a level 7mm above the obex, and only after a depth of 3-5mm had been reached.

2. This bradycardia was never produced if a midline incision in a rostrocaudal direction had been made previously.

DISCUSSION

Isolated Medulla Series

A. Basal heart rate

Because severing the spinal cord removes much sympathetic influence, the severe bradycardia which followed every spinal section was expected.

The usual increase in basal heart rate after a section at 8mm above the obex (through the rostral end of the auditory tubercles) is in agreement with the results of Hoff, Breckenridge and Spencer (1952). They found, with decerebrate dogs, that a section through the auditory tubercles partially relieved the bradycardia produced by a mid-pontine section. Results from both procedures indicate removal of a depressor region. But Alexander (1946) found that a similar section in cats with an otherwise intact nervous system evoked a depressor response and therefore, removal of a pressor area.

Since much sympathetic tone is removed in the isolated medulla preparation the rise in heart rate after the 8mm section probably results from elimination of some parasympathetic circuits. The depressor response of the intact animal may be due to removal of sympathetic pathways or "centers" above the mid-pontine region. If these pathways are a dominant factor in tonic control of the heart, then lesser parasympathetic influence would not be evident until the former pathways had been removed. The isolated medulla preparation gives indications that depressor fibers probably do exist at the caudal end of the pons though their influence is not great since only small increases in heart rate occur when they are severed.

The increase in heart rate at lower sections may be explained by assuming that progressively more parasympathetic circuits are being interrupted. However, this should be stated with reservations since these sections may possibly cause a gradual removal of a necessary reticular formation facilitatory system operating on medullary parasympathetic reflex centers.

It would be interesting to see the effect on basal heart rate, caused by cutting the vagi, after each of

these sections. The contribution to increased heart rate effected by removal of v agal motor pathways might then be demonstrated.

B. Change in heart rate with PDG

The PDG-induced bradycardia after decerebration and after spinal section was of similar magnitude in all animals except one. But this animal showed drastic changes in the PDG response at all transection levels (Table 2). The dosage of PDG always remained the same -- 100 µg because the drop in heart rate was not augmented by an increase of the drug. Since sympathetic tone is present in the decerebrate animal but considerably reduced after spinal section, PDG may act primarily by excitation of vagal effector mechanisms rather than by inhibition of sympathetic tone.

Since subsequent transections did not produce any drastic changes in the PDG response till a section through the rostral tractus solitarius, it is probable that the transections did not cut the region of primary sensory input or the final common vagal motor pathway. Thus, the area between the auditory tubercles and the rostral tractus would not be a primary parasympathetic reflex center. This is in agreement with the observations on

changes in basal heart rate after successive transections. The disappearance of the PDG response at this level is understandable if the secondary cardiovascular afferents arising from the nucleus of the tractus solitarius, synapse before or in the area just above the rostral portion of the tractus.

C. Duration of the PDG-induced bradycardia

The elimination of reverberating circuits, which are responsive to PDG, could account for the progressively diminishing duration of the PDG-induced bradycardia following each transection.

D. Effects of norepinephrine and stimulation of the carotid baroreceptors

Although these procedures were discontinued in later experiments, one point should be considered viz., the disappearance of the response to these operations was always simultaneous with the disappearance of the PDG response. This consistent coincidence can be explained on the hypothesis that peripheral stretch receptors act upon a common center for reflex responses to PDG and increased arterial pressure.

Histology

Although several cats in the first sub-series had lesions placed in the tractus solitarius and its nucleus,

no precise information about the paths of secondary afferents could be obtained. This was because the lesions involved cells and fibers from surrounding structures; consequently degenerated fibers with their origin in the sensory nucleus could not be separated from the profuse degeneration due to damage of other structures.

In the cats of the second sub-series also, there was an excessive amount of degeneration resulting from prolonged exposure to air, and to the multiple electrode penetrations. In the third sub-series the lesion was placed freehand to minimize damage to surrounding areas. However, only two cats with lesions in the tractus or its nucleus could be secured by this means. Of the pair, BC26 gave the most information about degeneration. In interpreting this information, four points should be considered:

First, degeneration was sparse. In several areas of the commissural nucleus there were sufficient degenerated fibres to be easily seen; but in the tractus and its nucleus the degenerated fibers were rare and very fine, while in the rostral area of the tractus degeneration was absent. The explanation may be that as the

lesion was small, only a few cells in the nucleus and only a few fibers in the tractus were destroyed.

Second, some of the degenerated fibers observed came from structures other than the tractus or its nucleus. For instance, the degenerated internal arcuate fibers probably originate in the gracile nucleus. Damaged fibers from this nucleus were seen to curve laterally and ventrally towards the arcuate complex; and Papez (1929) has shown that the gracile nucleus gives rise to the internal arcuate fibers which in turn constitute the medial lemniscus. Since some degeneration was seen also in the medial leminscus it is reasonable to assume that neither its fibers nor those of the internal arcuate complex are secondary cardiovascular afferents.

Third, since degenerated fibers were seen in the nucleus intercalatus, following damage to the nucleus solitarius, it is possible they were secondary cardiovascular afferents. Crosby and Woodburn (1951) provide further evidence; they state that the medial portion of the dorsal fasciculus of Schutz lies in close relationship to the dorsal motor nucleus of the vagus, and to the hypoglossal nucleus; thus they place it close to the

intercalate nucleus. They also state that the nucleus solitarius contributes ascending fibers to the dorsal fasciculus.

Fourth, a good many secondary cardiovascular afferents may synapse below the level of the rostral third of the tractus. The evidence is as follows:

1. The response to PDG always disappeared with a section just below the rostral third of the tractus, but above, or including only the rostral tip of the dorsal motor nucleus of the vagus. Thus, the transection could have severed connections between secondary cardiovascular afferents and higher parasympathetic "centers". Another feasible alternative could be that the progressively more caudal transections severed descending pathways providing essential facilitation to more caudal vagal synapses. Care was taken that in each cat all the Xth rootlets and most of the IXth rootlets were not severed. After entering the medulla, the vagal fibers turn rostrally before taking a caudal direction in the tractus solitarius. However, few if any of these primary afferents were severed by the final transection. This transection was approximately level with the rostral end of the olivary nucleus. Ranson and Clark (1959) show very few vagal fibers at this level.

2. Following damage to the tractus solitarius, degeneration was seen below, but not above the level of the rostral third of the tractus.

Fifth, no degeneration was seen in the dorsal motor nucleus of the vagus. This is in agreement with the findings of Cottle (1964). It suggests a multisynaptic pathway from the nucleus of the tractus to the dorsal motor nucleus, if the latter is the cell station for the final common vagal pathway.

Partial Transection Experiments

The bradycardia produced after a partial transection to the left of the mid-line at 7mm above the obex might be evidence of ventral depressor pathways since the response did not occur until a depth of 3-5mm had been reached. It cannot be said whether this area contains vagal second order afferents because no degeneration was traced there. The failure to produce bradycardia in this area after a mid-line incision indicates that rostro-caudal affector pathways for bradycardia are crossed.

The rise in heart rate after a section at 3mm above the obex on either side would be expected since this transection eliminates the upper half of the tractus solitarius and also some of the dorsal motor nucleus of the vagus.

SUMMARY

Twenty cats were subjected to decerebration and spinal section to obtain information about the reactions of the isolated medulla to various physiological procedures. The objective was to elucidate and describe the organization of parasympathetic pathways involved in cardiomotor regulation.

Cats in this series showed a successive increase in heart rate as the brain stem was transected in a rostro-caudal direction along with a disappearance of the PDG reflex bradycardia following a section through the rostral one-third of the tractus solitarius. This increase in heart rate may indicate the successive removal of essential mechanisms facilitating more caudal vagal synapses. This transection also produced a disappearance of the secondary bradycardia due to norepinephrine.

Lesions were placed in cats allowed to recover from aseptic brain stem exposure, in the tractus solitarius or its nucleus, so that secondary cardiovascular afferents could be identified by their degeneration. The two cats which yielded usable results showed degeneration within

the nucleus of the tractus solitarius and within adjacent structures, such as the gracile nucleus and the nucleus intercalatus. Degeneration in these areas and in the ventral commissure of the cord terminated in the region of the rostral tractus solitarius. Of these degenerated fibers, only those within the nucleus intercalatus could be considered secondary cardiovascular afferents.

The absence of degeneration above the level of rostral tractus solitarius together with the disappearance of the PDG reflex at that level indicates that some cardiovascular reflex synaptic sites occur in this area but that their operation is modified by more rostral connections.

Figure 1

Transverse section through the rostral one-third of the tractus solitarius showing level of transection which abolished the response to PDG; arrow points to region of the tractus solitarius. 50μ thick, Kernechtrot stain, Cat IMP 13.


1

Figure 2

Diagram indicating levels of transections performed on all cats in the isolated medulla series. Thickness of arrows indicates range of transections at each "level".

Key

FN = Facial nucleus

TSN = Trigemino-spinal nucleus

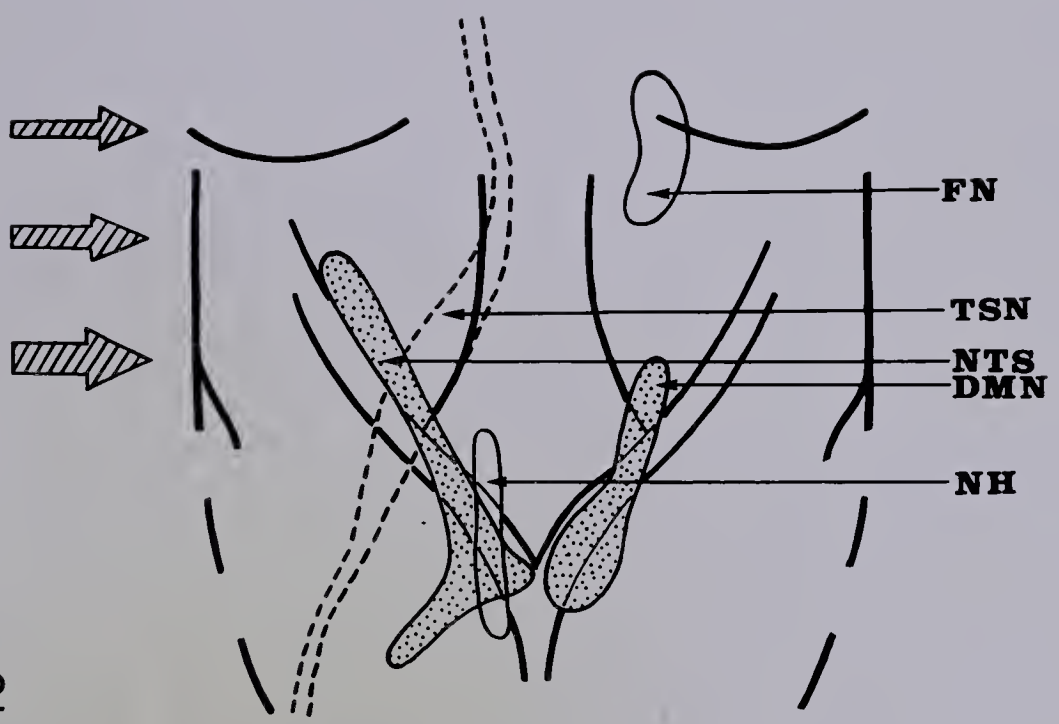
NTS = Nucleus of the tractus solitarius

DMN = Dorsal motor nucleus of the vagus

NH = Hypoglossal nucleus

Figure 3

Dots in diagram indicate the stimulation lesion sites, in the region of the tractus solitarius, for all cats in histological Sub-Series A.



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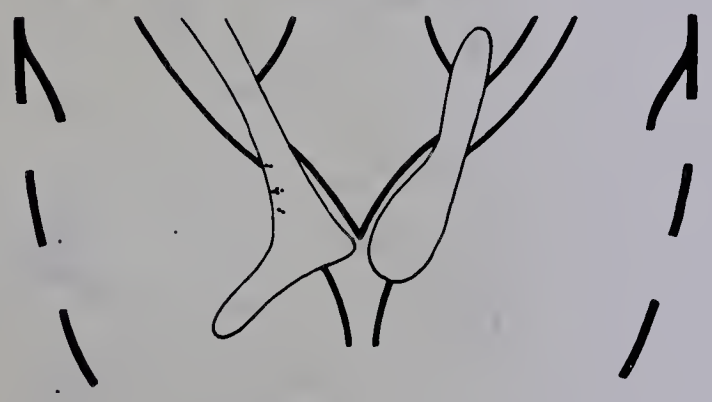

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Figure 4

Transverse section through medulla showing large lesion in region of the vagal sensory nucleus in Sub-Series A cat. 35μ thick, Nauta stain, Cat BC4.x 120

Figure 5

Transverse sections through medulla, showing control lesions produced using different time and duration combinations.

(a) .1V for 6 seconds

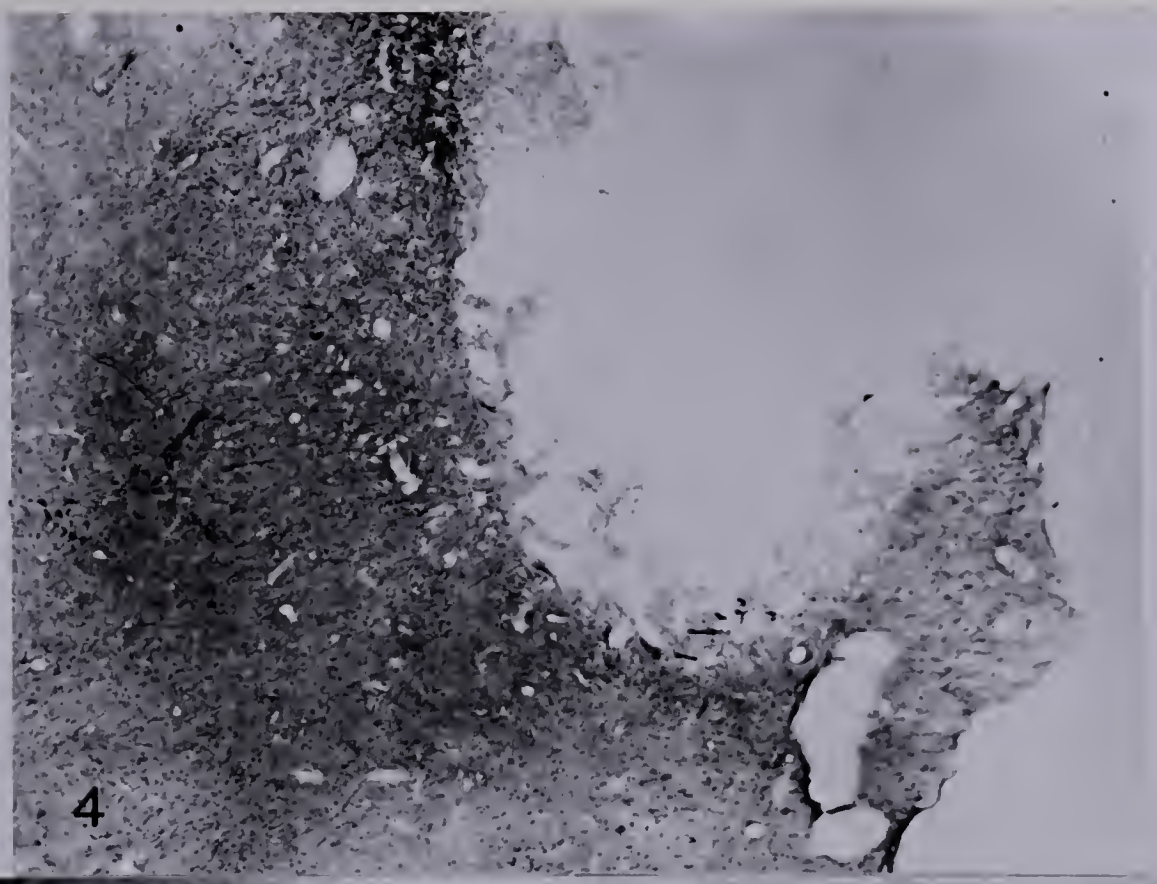
(b) .25V for 1-2 seconds

(c) .04V for 3 seconds

35μ thick, Kernechtrot stain, Cat BC 19.x 120

Figure 6

Transverse section showing lesion in the gracile nucleus from a Sub-Series B cat. Profuse degeneration surrounding lesion is due mostly to exposure of brain stem and multiple electrode penetrations, 35μ thick, Nauta stain, Cat BC 19. x120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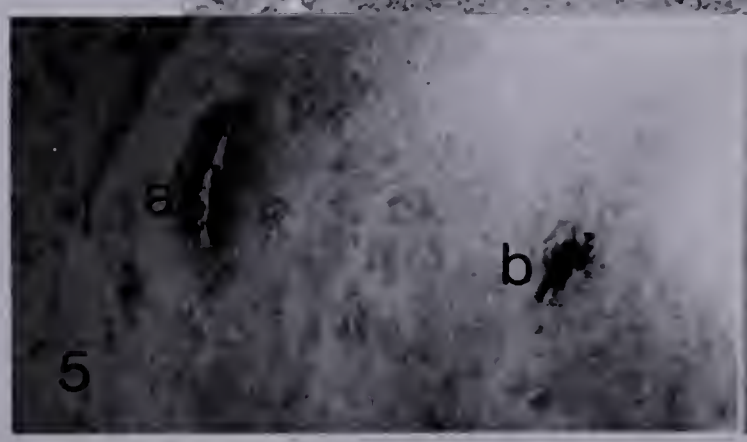
a
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c
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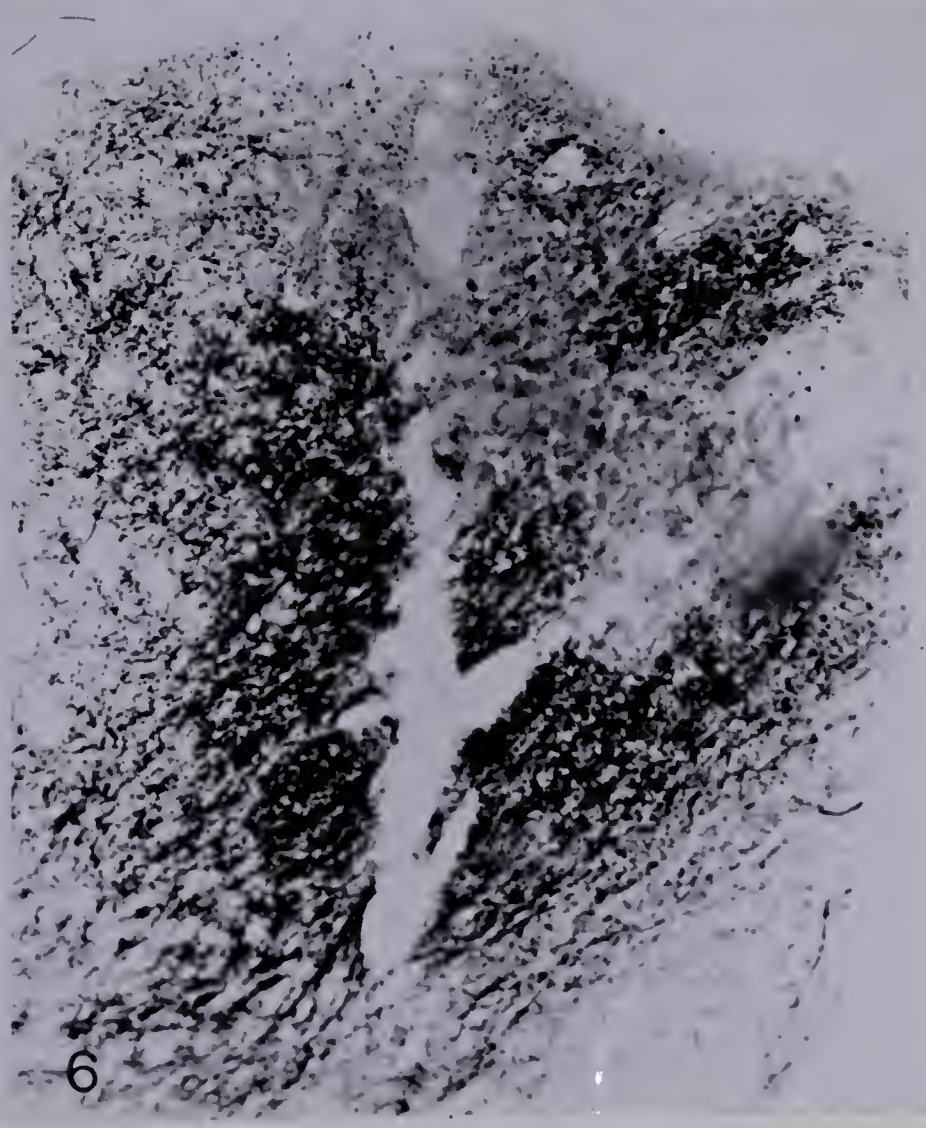
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Figure 7

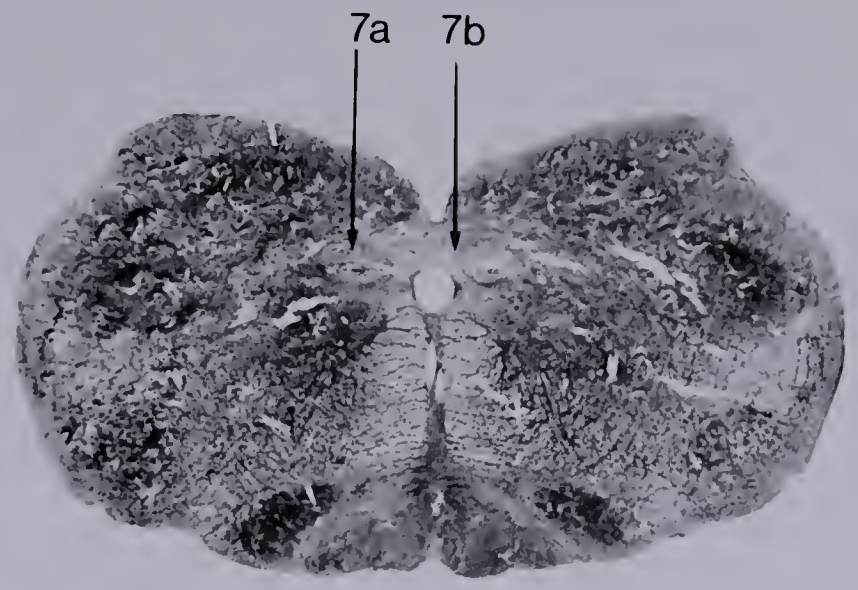
Transverse section just below level of obex to indicate the region of degenerating fibers shown in figs. 7a and 7b. 35μ thick, Nauta stain, Cat BC 26. x 7.5

Figure 7a

Degenerating fibers in the nucleus of the tractus solitarius at the level and region indicated by the arrow in fig. 7. 35μ thick, Nauta stain, Cat BC 26. x 1300

Figure 7b

Degenerating fibers in the commissural nucleus of Cajal, the midline connecting component of the nucleii solitarii at the level and region indicated by the arrow in fig. 7. 35μ thick, Nauta stain, Cat BC 26. x 500



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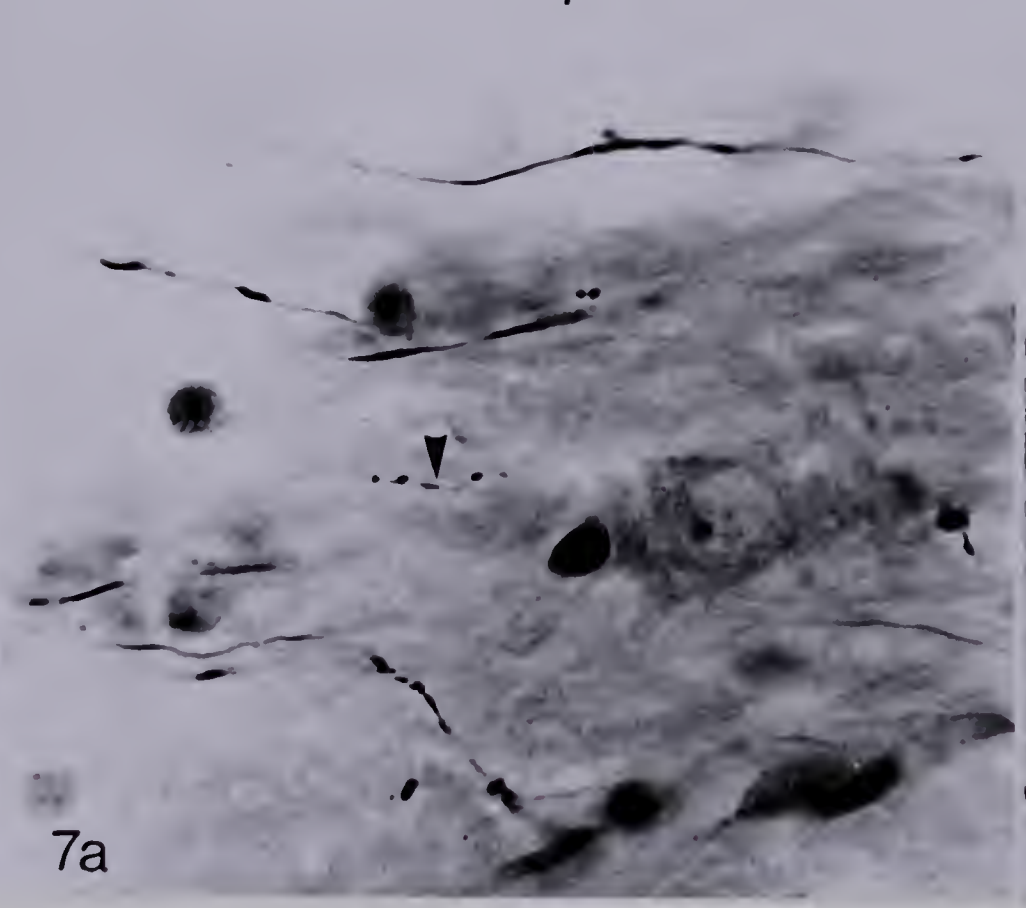


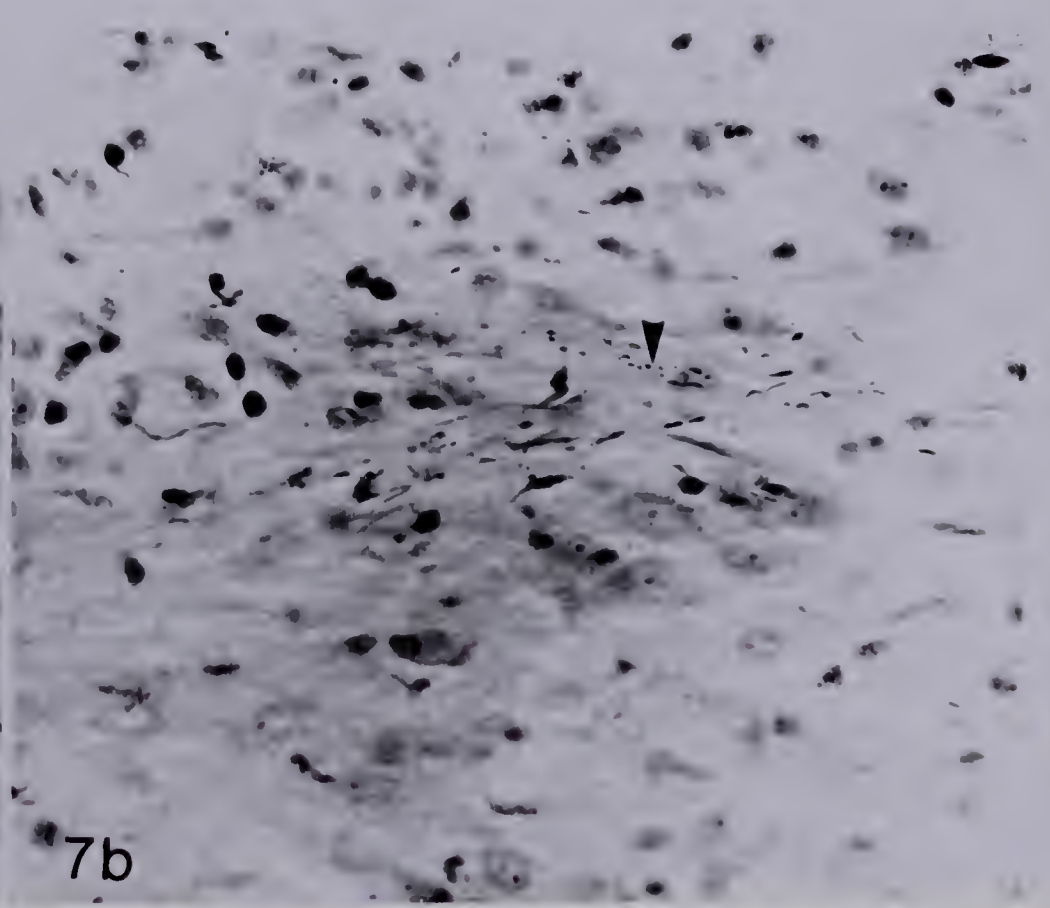


Figure 8

Transverse section just below level of obex to indicate the region of degenerating fibers shown in figs. 8a and 8b. 35 μ thick, Nauta stain, Cat BC 26. x 7.5

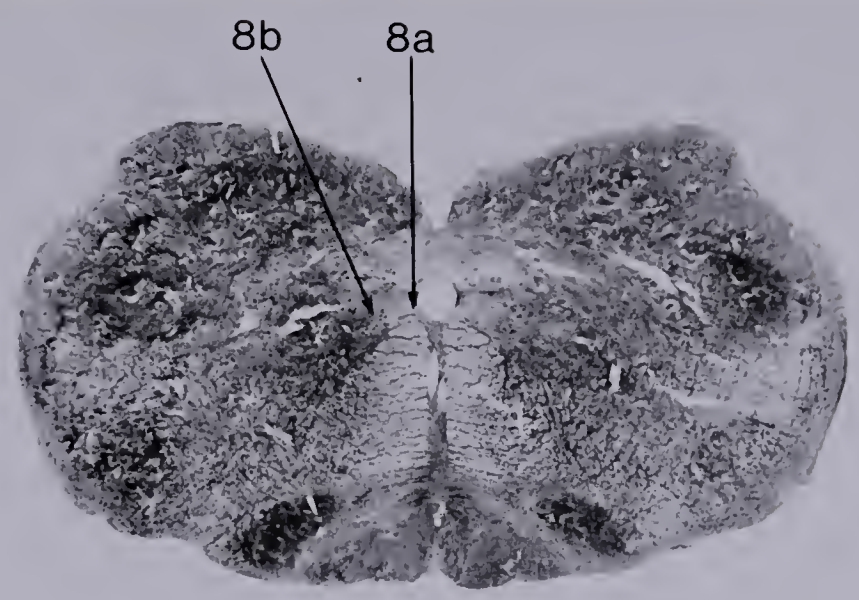
Figure 8a

Degenerating fibers in the ventral commissure of the cord at the level and region indicated by the arrow in fig. 9. 35 μ thick, Nauta stain, Cat BC 26. x 500

Figure 8b

Degeneration in the nucleus intercalatus at the level and region indicated by the arrow in fig. 8.

35 μ thick, Nauta stain, Cat BC 26. x 500



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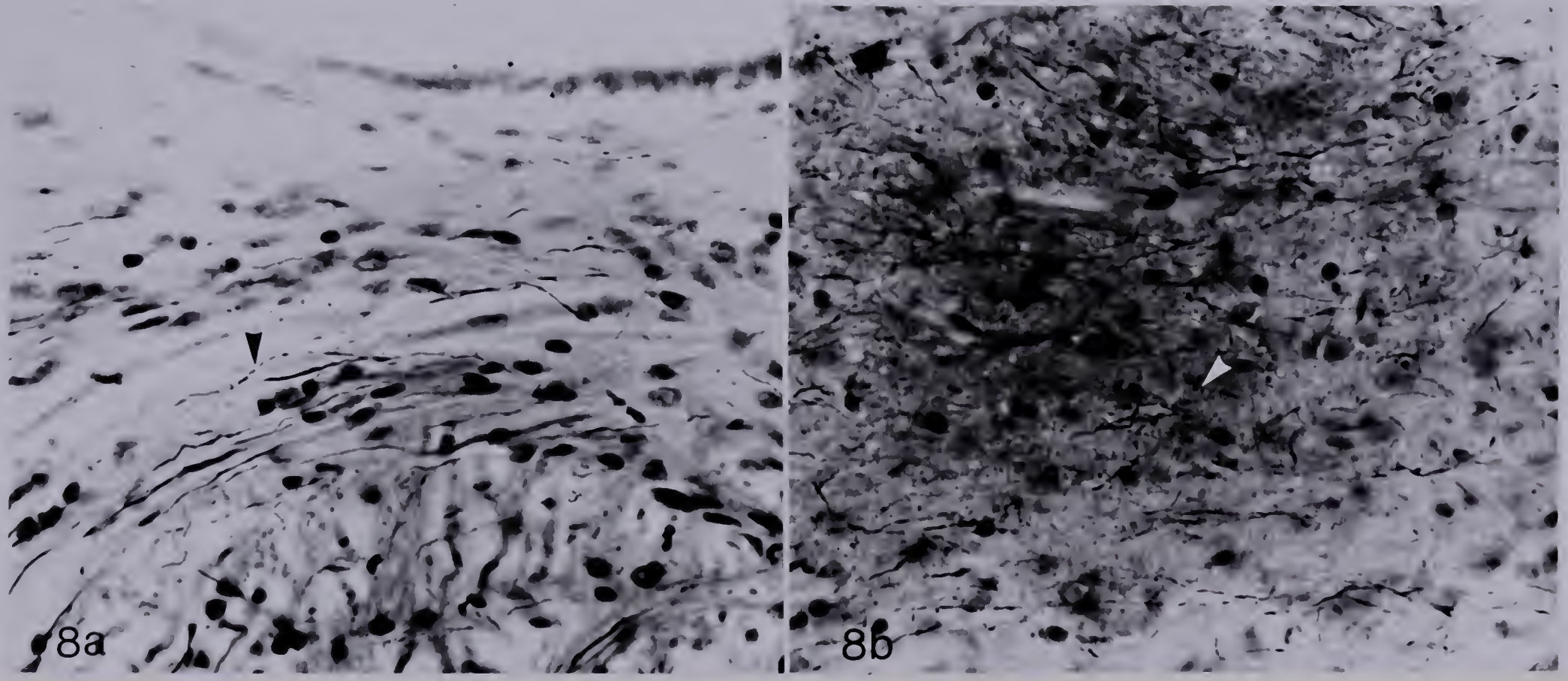


Figure 9

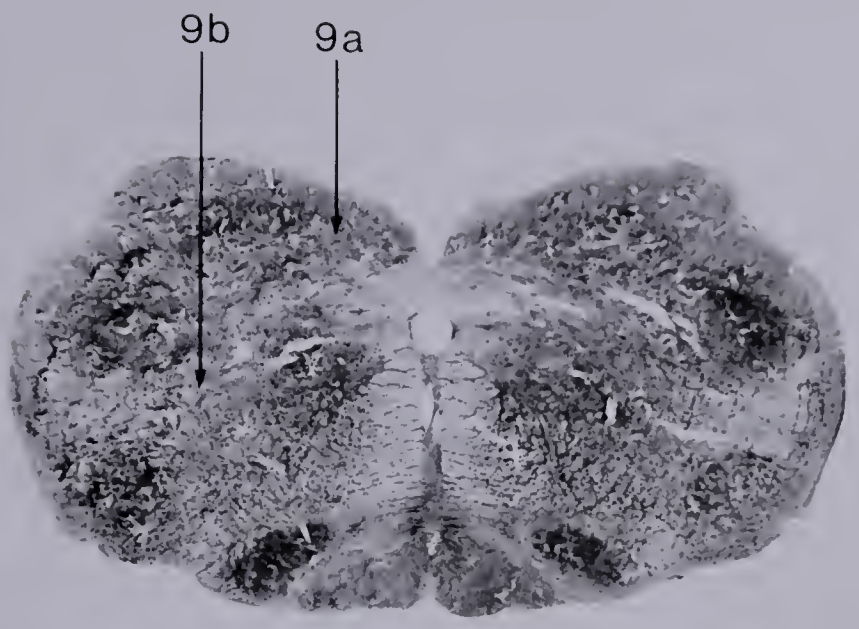
Transverse section just below the level of the obex to indicate the region of degenerating fibers shown in figs. 9a and 9b. 35μ thick, Nauta stain, Cat BC 26. x 7.5

Figure 9a

Degeneration in the gracile nucleus at the level and region indicated by the arrow in fig. 9. 35μ thick, Nauta stain, Cat BC 26. x 500

Figure 9b

Degenerating internal arcuate fibers as a result of damage to the gracile nucleus. The level and region are indicated by the arrow in fig. 9. 35μ thick, Nauta stain, Cat BC 26. x 1300



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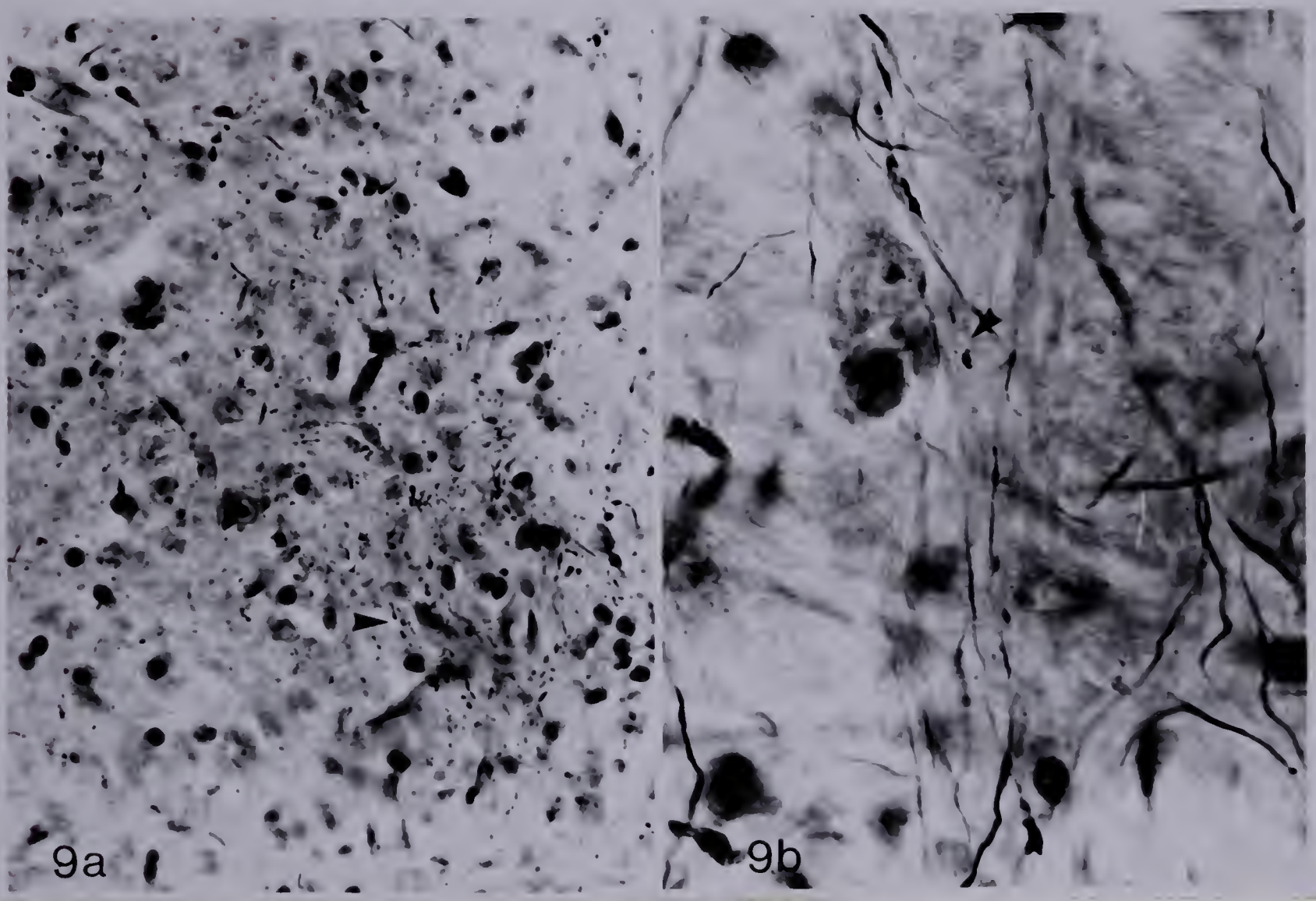
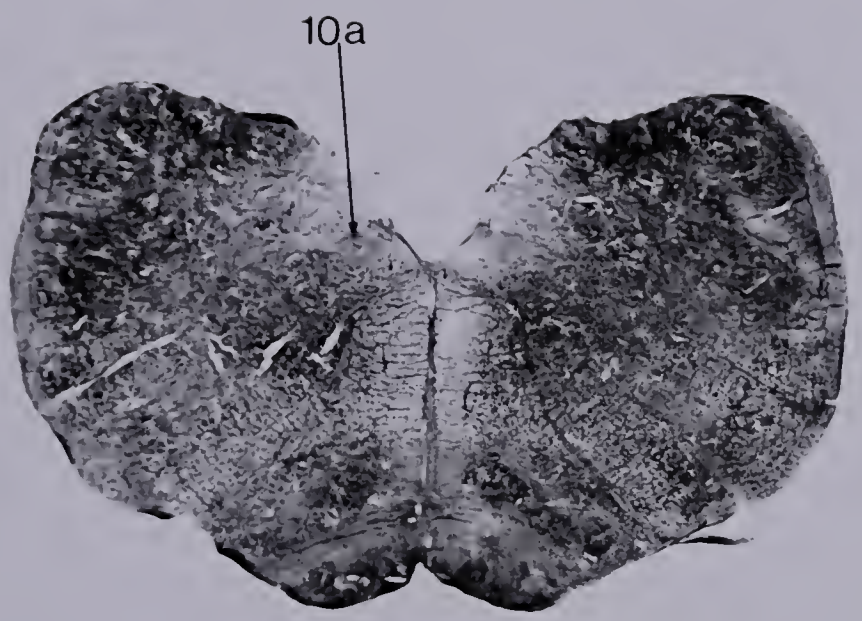


Figure 10

Transverse section at the level of the middle of the olivary nucleus to indicate the region of degenerating fibers shown in fig. 10a. 35/4 thick, Nauta stain, Cat BC 26. x 7.5

Figure 10a

Degeneration in the intercalate nucleus at the level and region indicated by the arrow in fig. 10. 35/μ thick, Nauta stain, Cat BC 26. x 500



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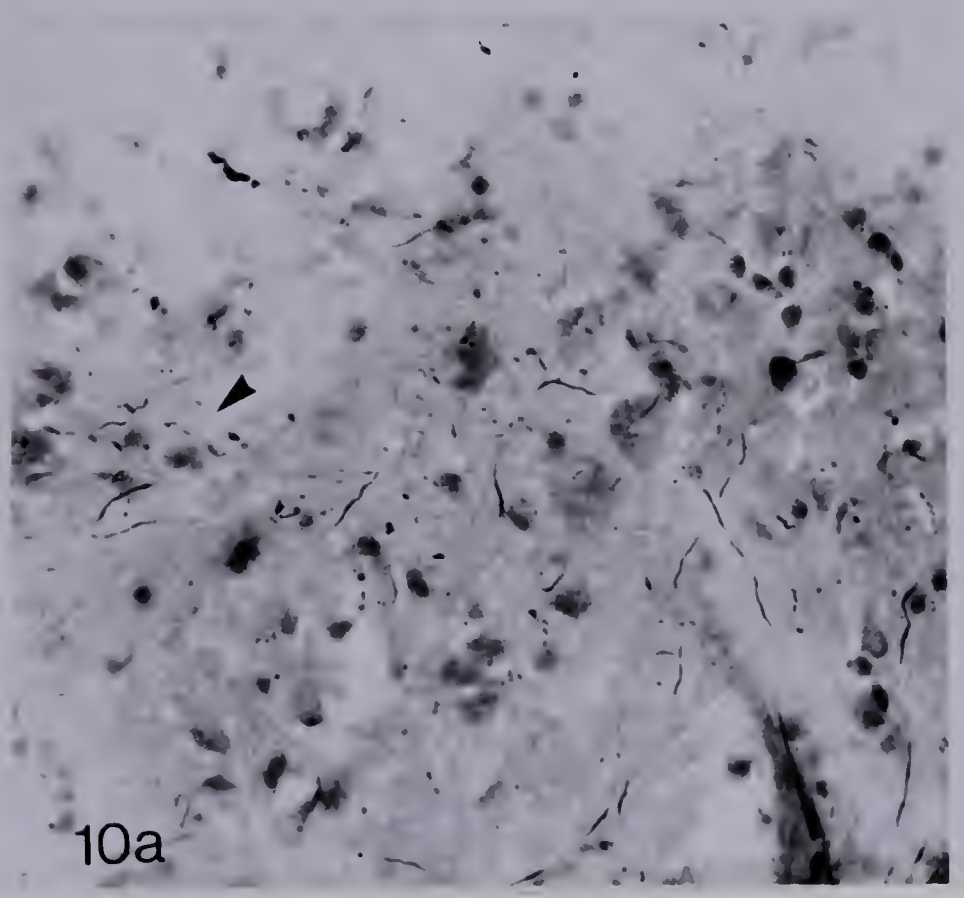

BIBLIOGRAPHY

1. Alexander, R. S.: Tonic and reflex functions of medullary sympathetic cardiovascular centers. J. Neurophysiol. 9: 205, 1946.

2. Bach, L. M. N.: Relationships between bulbar respiratory, vasomotor and somatic facilitatory and inhibitory areas. Am. J. Physiol. 171: 417, 1952.

3. Cottle, M. K.: Degeneration studies of primary afferents of 1Xth and Xth cranial nerves in the cat. J. Comp. Neur. 122: 329, 1964.

4. Crosby, E. C., and Woodburne, R. T.: The mammalian midbrain and isthmus regions Part II. The fiber connections C. The hypothalamo-tegmental pathways. J. Comp. Neur. 94: 1, 1951.

5. Dell, P., and Olson, R.: Projections "secondaires" naesencephaliques, diencephaliques et amygdaliennes des afferences viscerales vagales. C. R. Soc. Biol. (Paris). 145: 1084, 1951.

6. Foley, J. O., and Dubois, F. S.: An experimental study of the rootlets of the vagus nerve in the cat. J. Comp. Neur. 60:137, 1934.

7. Folkow, B., Johansson, B., and Oberg, B.: A hypothalamic structure with a marked inhibitory effect on tonic sympathetic activity. Acta Physiol. Scand. 47: 262, 1959.

8. Gerebetzoff, M. A.: Les voies centrales de la sensibilite et du gout et leurs terminaisons thalamiques. La Cellule. 48: 91, 1939.

9. Glasser, R. L.: Vagal inhibition of cardiovascular activity in the decerebrate cat. Am. J. Physiol. 203:449, 1962.

10. Green, J. D.: A simple microelectrode for recording from the central nervous system. Nature. 182:962, 1958.

11. Hoff, H.E., Breckenridge, C. G., and Spencer, W. A.: Suprasegmental integration of cardiac innervation. Am. J. Physiol. 171:178, 1952.

12. Ingram, W. R., and Dawkins, E. A.: The intramedullary course of afferent fibers of the vagus nerve of the cat. J. Comp. Neur. 82:157, 1945.

13. Lindgren, P., and Uvnäs, B.: Vasodilator responses in the skeletal muscles in the dog to electrical stimulation in the oblongate medulla. Acta Physiol. Scand. 29:137, 1953.

14. Lindgren, P., and Uvnäs, B.: Postulated vasodilator center in the medulla ablongata. Am. J. Physiol. 176:68, 1954.

15. Lindgren, P., Rosen, A., Strandberg, P., and Uvnäs, B.: The sympathetic vasodilatator outflow. A cortico-spinal autonomic pathway. J. Comp. Neur. 105:95, 1956.

16. Manning, J. C.: Intracranial representation of cardiac innervation. In: Nervous control of the Heart, Ed. by W. C. Randall, the Williams and Wilkins Co., Baltimore, Md. pp. 16-33, 1965.

17. Nauta, W. J. H.: Silver impregnation of degenerating axons. In: New Research Techniques of Neuroanatomy, Ed. by W. F. Windle, Charles C. Thomas, Springfield, Ill., pp. 17-26, 1957.

18. Owsjannikow, P.: Die tonischen und reflectoreschen centren der gefassnerven. Ber. Sachs. Ges. Wiss., math-phys. K123:135, 1871.

19. Papez, J. W.: Comparative Neurology, Thomas Y. Crowell Co., New York, 1960.

20. Peiss, C. N.: Central control of sympathetic cardioacceleration in the cat. J. Physiol. 151:225, 1960.

21. Porter, R.: Unit responses evoked in the medulla oblongata by vagus nerve stimulation. J. Physiol. 168:717, 1963.

22. Ranson, S. W., and Billingsley, P. R.: Vasomotor reactions from stimulation of the floor of the fourth ventricle. Studies in vasomotor reflex arcs. III Am. J. Physiol. 41:85, 1916.

23. Ranson, S. W., and Clark, S. L.: The Anatomy of the Nervous System, W. B. Saunders Co., Philadelphia, Penn., 1960. p. 476.

24. Reis, D. J., and Cuenod, M.: Central neural regulation of carotid baroreceptor reflexes in the cat. Am. J. Physiol. 209:1267, 1965.

25. Rosen, A.: Augmentation of cardiac contractile force and heart rate by medulla oblongata stimulation in the cat. Acta physiol. scand. 53:255, 1961.

26. Salmoiraghi, G. C.: "Cardiovascular" neurones in brain stem of cat. J. Neurophysiol. 25:182, 1962.

27. Schwartz, H. G., Roulhac, G. E., Lam, R. L. and O'Leary, J. L.: Organization of the fasciculus solitarius in man. J. Comp. Neur. 94:221, 1951.

28. Scott, J. M. D.: The part played by the ala cinerea in vasomotor reflexes. J. Physiol. 59:443, 1925.

29. Taber, E.: The cytoarchitechure of the brain stem of the cat. I. Brain stem nuclei of cat. J. Comp. Neur. 116:27, 1961.

30. Thompson, W. C., and Bach, L. M. N.: Some functional connections between hypothalamus and medulla. J. Neurophysiol. 13:455, 1950.

31. Torvik, A.: Afferent connections to the sensory trigeminal nuclei, the nucleus of the solitary tract and adjacent structures. An experimental study in the rat. J. Comp. Neur. 106:51, 1956.

32. Tuttle, R. S., and Connor, R. S.: An intense hypotensive response evoked by stimulation in the pyramidal cat. Am. J. Physiol. 208:693, 1965.

32. Urabe, M., and Tsubokawa, T.: Distribution of activating neurons in medulla oblongata by stimulation of the vagus nerve. Neurologica medico-chirurgica. 2:147, 1960.

34. Wang, S. C., and Ranson, S. W.: Autonomic responses to electrical stimulation of the lower brain stem. J. Comp. Neur. 71:437, 1939.

35. Calaresu, F. R., and Pearce, J. W.: Effects on heart rate of electrical stimulation of medullary vagal structures in the cat. J. Physiol. 176: 241, 1965.